6. DISCUSSION

Capecitabine remains an important therapeutic strategy in colorectal cancer patients due to its oral formulation, ease of dose modifications and administration in patients with liver dysfunction, and the lack of cumulative toxicity following long periods of administration. However, in clinical practice, the FDA recommended dose of 2,500 mg/m\(^2\) given in two divided daily doses for 14 days followed by 7 days of rest [139]. Literatures support a severe grade 3 toxic effect of capecitabine [140]. Therefore, a starting dose of 1,000 mg/m\(^2\) is commonly used in clinical practice with similar apparent efficacy.

The pharmacokinetic profile of capecitabine and its metabolite 5 FU observed in our study was highly variable between patients but was similar to previous findings [141,142]. Extensive metabolism of capecitabine contribute to high inter patient variability in the C\(_{\text{max}}\) and AUC of the parent as well as its metabolite 5FU. [143]

Since aging is the result of highly individualized processes, a sub group analysis of our study, demonstrates that age did not have any impact on the safety and efficacy profile of capecitabine. This was Similar to a study by Søren Astrup Jensen et al were in the efficacy and toxicity of palliative chemotherapy for elderly and younger colorectal cancer patients were compared and reported that elderly patients had similar frequencies of grade 3 or 4 toxicity as compared to as younger patients [144]. However, Capecitabine should be avoided in elderly patients with renal dysfunction or overlapping toxicities.
A study by S G Louie et al indicated a notable increase in capecitabine area under the curve (AUC) but no statistical difference found with the active moiety, 5FU [145]. However in our study no statistical difference in AUC of capecitabine and 5 FU was observed among the elderly. Suggesting that metabolic enzymes involved in converting capecitabine metabolites are not altered by age.

The cumulative results of evidence, indicates that the clearance of capecitabine in women was less than that in men. This may possibly explain more toxicity among female as compared to males. Alastair Ilich et al showed that Females had a significantly higher dose-limiting toxicity incidence than males (67.7 vs. 52.2%, p = 0.007) with a dose of 1250mg/m$^2$, whereas a study by Hennessey et al. reported that a dose of 1000 mg/m$^2$ produced less toxicity, required less frequent dose reduction and achieved similar efficacy in their population when compared to 1250 mg/m$^2$ dosing among females[146,147]. In our study male had higher incidence of toxicities as compared to females and males where better responders which is also reflected by higher C$_{max}$ among male as compared to females, indicating higher enzyme activity among male as compared to female. Studies have also shown that toxicity may predict a good response to chemotherapy [148,149].

A study findings from Hany Elsaleh et al reported that men with right-sided tumours benefited from chemotherapy compared to men with left-sided tumors[150]. The features seen predominantly in left sided cancers such as mutant p53, and over expression of vascular endothelial growth factor are associated with an adverse prognosis and poor response to fluorouracil based chemotherapy. But in
our study there was no statistically significant difference in response between left and right sided cancers which is also evident with the pharmacokinetic parameters of Capecitabine and 5FU.

As the colorectal cancer metastasize to the liver, hepatic dysfunction is relatively common in patients. Twelves et al. Correlated the degree of hepatic dysfunction in patients who had metastatic colorectal cancer with calculated pharmacokinetic parameters for capecitabine[151]. The $C_{\text{max}}$ and the AUC for the main metabolites of capecitabine were elevated to some extent in patients with mild to moderately impaired liver function, but not sufficiently so to warrant a recommendation of dose reduction. However in our study there was no statistically significant difference in the pharmacokinetic parameters of capecitabine and 5 fluorouracil among the mild to moderately impaired liver function. Indicating that mild to moderate hepatic impairment does not affect the bioactivation of Capecitabine. The patients with normal hepatic function were better responders with less grade 3 global toxicity as compared to patients mild or moderate dysfunction.

In our study, renal dysfunction had no impact on capecitabine disposition when patients with normal renal function were compared with subjects with mild, moderate renal dysfunction which was similar to a study by Poole et al, 2002 as urinary excretion is a minor pathway of elimination for 5-FU[152].
Promising pharmacogenetic data have been reported from the analysis of \textit{TS}, \textit{DPD}, and \textit{MTHFR} gene polymorphisms, which could explain the variation in response or toxicity in cancer patients receiving 5-FU–based chemotherapy and Ethnic diversity in drug response or toxicity is becoming increasingly recognized as an important factor accounting for inter-individual variation in anticancer drug responsiveness [153-155].

Thymidine synthase (\textit{TS}) plays an important role in DNA replication. In \textit{TS} gene, the promoter region is polymorphic, having either a double repeat (2R) or triple repeat (3R) of a defined 28bp sequence. The present study, suggests that patients bearing 2R/3R TS genotype were not good responders for the therapy which is similar to a study by Remy Largilllier et al were in the authors have reported a lower response rate to capecitabine in patients bearing the TS genotype associated with higher TS expression (i.e., 3R3R) relative to 2R2R [156]. It must be borne in mind that intracellular TS expression may have a dual role in tumor evolution. As a 5-FU target, high TS expression is related to 5-FU resistance [157]. On the other hand, elevated TS expression reflects tumor aggressiveness and can also be an indicator of unfavorable prognosis [158].

Two widely studied polymorphisms which diminish the activity of the \textit{MTHFR} gene, namely 1298A>C (rs1801131) and 677C>T (rs1801133). Even though the impact of MTFHR genotype on tumoral CH2FH4 (methylenetetrahydrofolate) concentrations has not been clearly established, deficient MTHFR genotypes may theoretically favour an increase in intracellular methylenetetrahydrofolate concentrations.
MTHFR 677C>T is one of numerous polymorphisms of the MTHFR gene described in the literature, which may contribute to activity changes in this enzyme. MTHFR 677TT genotype is responsible for a 30% reduction in enzymatic activity compared to 677CC genotype associated with reduced thermolability observed in vitro, which results in a decreased erythrocyte concentration and accumulation of CH2FH4 [159]. This may have a significant effect on the pharmacological efficacy of 5-FU. The 5-FU and 5-FdUMP metabolite irreversibly forms a stable complex with TS and CH2THF. Creation of this complex inhibits the activity of TS, which leads to an intracellular drop in dTMP concentration and finally inhibition of DNA synthesis. Increased concentration of CH2THF as a consequence of the presence of the MTHFR 677C>T polymorphism may therefore contribute to changes in the chemosensitivity of cancer cells exposed to 5-FU by increasing the amount and stability of CH2THF-TS-FdUMP ternary complex, and thus a stronger inhibition of DNA synthesis. Sohn et al[160] in both in vitro and in vivo studies observed that the presence of 677T allele of the MTHFR gene is responsible for greater chemosensitivity in colon cancer cells, suggesting that the genetic variant 677C>T may be a pharmacogenetic factor used to assess the effectiveness of 5-FU-based chemotherapy. In advanced CRC patients undergoing 5-FU-based therapy, in three published studies the presence of the 677T variant of the MTHFR gene was associated with a higher percentage of positive responses [161-163], while the results of another study did not confirm the existence of such a relationship [164]. There was no relationship of 677TT variant with response observed among our study population as well.
Similarly MTHFR1298A>C polymorphism contributes to the reduction in enzymatic activity of MTHFR. Some of the published studies on SNP 1298A>C suggest that the presence of the 1298_c variant of the MTHFR gene has no impact on the percentage of positive responses to 5-FU treatment[163-165], while two studies suggest that it is associated with significantly decreased patient survival time[166,167].

Capitain et al. found that 1298A>C but not 677C>T was associated with increased toxicity in patients with metastatic CRC (mCRC) treated with 5-FU/leucovorin [168]. Tsunoda et al. showed that AA carriers with the 1298A>C suffered less fatigue than AC carriers, although no association with the 677C>T SNP was observed[169]. In this sense, Sharma et al. found that patients with the AA genotype for 1298A>C experienced fewer grade 2 or higher ADRs when treated with capecitabine [170]. By contrast, other studies have found that the 677C>T SNP but not 1298A>C are associated with grade 3–4 toxicity and diarrhea [171-173]. Other authors found that CC genotypes correlated with less toxicity [174,175]. Furthermore, several studies have been unable to show an association between any of these polymorphisms and toxicity [176]. In subgroup analysis Loganayagam et al. reported MTHFR12 98CC homozygous variant genotype as a predictive marker for hand–foot syndrome [177]. Our study also showed no statistical difference between the variants of 677C>T and 1298A>C SNPs with toxicity.

Thirty-nine different mutations and polymorphisms leading to decreased enzyme activity with DPD and therefore increased risk of toxicity has been
observed [178]. The most frequent inactivating mutation is IVS14+1G>A leading to skipping exon 14 and therefore missing 165 nucleotide in mRNA and the corresponding 55 amino acids in the protein product. Mutation and polymorphism in DPD resulting in decreased degradation of 5FU from capecitabine is associated with severe toxicity. In our study, IVS14+1G>A mutation was found in only one case in heterozygote state, with Grade 3 global toxicity and poor response. Severe toxicities following exposure to 5-FU or the 5-FU oral analog, capecitabine, were reported at higher rates in patients who are heterozygous (possessing two different forms of the gene) for the mutant DPD allele, compared with patients who are homozygous (possessing two identical forms of the gene) for the wild-type, or unmutated allele [179]. However, there was no correlation between standard pharmacokinetic parameters of capecitabine and 5FU with the genetic variation. This could be possibly explained by the assessing the influence of the degraded metabolite of 5FU.

The final population pharmacokinetic model revealed no clinical relevance. The base model was developed and the influence of the covariates was also carried out. The covariate effect on the pharmacokinetics of capecitabine was consistent with a study by Gieschke R et al among breast cancer patients [180].